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Customer No.: **07278**

Docket No: 01728/100F088-US2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Ick-Dong Yoo, et al.

Serial No.: 09/846,634 Art Unit: 1651

Confirmation No.: 5673

Filed: May 1, 2003 Examiner: Vera Afremova

For: NOVEL IMMUNO-STIMULATING POLYSACCHARIDE SUBSTANCE FROM PHELLINUS SPP. STRAIN AND USE THEREOF

DECLARATION PURSUANT TO 37 C.F.R. §1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

June 5, 2003

I, Ick-Dong Yoo, hereby declare as follows:

1. I am a citizen of the Republic of Korea and I am more than 21 years of age.

2. I am one of the named invent rs in the above-captioned patent application, U.S. Serial No. 09/846,634. I make the following averments for myself and on behalf of my co-inventors.

3. The experiments described below verify that the immuno-stimulatory capability of PL extract isolated from KCTC 0399BP is higher than that of other *Phellinus linteus* strains, including ATCC 26710, 0173BP, and L13202. This provides for a more efficient use in immuno-stimulatory regimens due to the superior immuno-stimulating capabilities of the compositions recited in the claims.

4. **Comparative $CD8^+$ T-Lymphocyte-Stimulating Activity:**

The ability of the immuno-stimulating substance isolated from KCTC 039BP to stimulate $CD8^+$ T-lymphocytes was compared to that of immuno-stimulating substances, *i.e.*, "PL extracts" (see pages 27-28 of the specification, Example X), isolated from *Phellinus linteus* strains ATCC 26710, 0173BP, and L13202. Strain 0173BP is the strain described by Lee et al, *The Korean Journal of Mycology*, Vol. 23, No.4, pp. 325-331 (Dec. 1995) and L13202 is the strain described by KR 97-15743. As controls, no substance, PBS, or Concavalin A (ConA) were used. Basically, this test was carried out as outlined in Example XI (pages 28 to 30 of the specification) and Example XIV (pages 31 to 32 of the specification), although Fluorescence-Activated Cell-Sorting (FACS) analysis was used for detection of $CD8^+$ lymphocytes.

5. Briefly, splenocytes were isolated from female Balb/c mice. Cell suspensions were then added to 6-well plates at a concentration of 2×10^6 cells/ml.

Next, PL extract (100 µg/ml) or control (5 µg/ml ConA) was added (one per well) to the wells. The cells were then incubated for 7 days at 37°C in a CO_2 incubator before harvesting, and $CD8^+$ T-lymphocytes detected using FACS analysis and a flow cytometer.

6. The results, depicted in Figure A below, showed that the immuno-stimulatory substance isolated from KCTC 0399BP resulted in a higher T-lymphocyte activation than those isolated from the other *Phellineus linteus* strains as well as the controls.

CD8 T-lymphocytes Activity of 0399BP (FACS analysis)

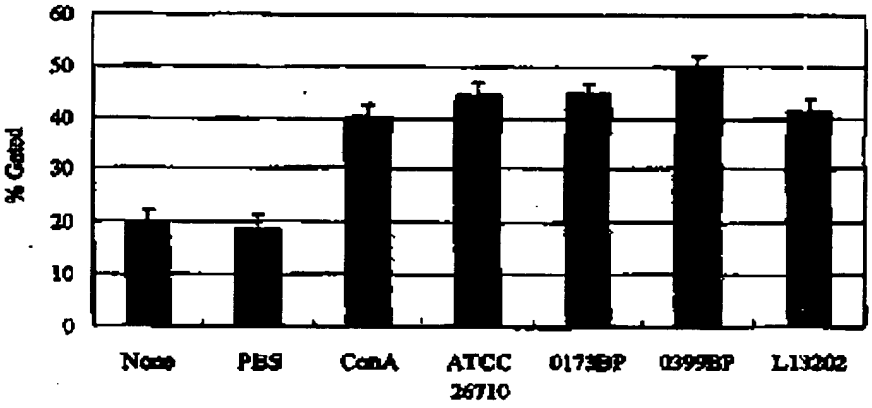

FIGURE A

7. **Comparative B-Lymphocyte-Stimulating Activity:**

The ability of the immuno-stimulating substance isolated from KCTC 0399BP to stimulate B-lymphocyte activity was compared to that of immuno-stimulating substances, "PL extracts", isolated from *Phellinus linteus* strains ATCC

26710, 0173BP, and L13202. Again, strain 0173BP is the str in described by Lee et al, *The Korean Journal of Mycology*, Vol. 23, No.4, pp. 325-331 (Dec. 1995) and L13202 is the strain described by KR 97-15743. As controls, no substance, PBS, or lipopolysaccharide were used. Basically, this test was carried out as outlined in Example XI (pages 28 to 30 of the specification) and Example XIV (pages 31 to 32 of the specification), using an Antibody-Forming Cells (AFC) assay based on haptenation of sheep red blood cells (sRBC).

8. Briefly, splenocytes were isolated from female BDF1 mice. Cell suspensions were then added to 48-well plates at a concentration of 5×10^6 cells/ml, in triplicate. Next, PL extract (100 µg/ml) or control (LPS 25 µg/ml) was added (one per well) to the wells. The cells were then incubated for 3 days in an atmosphere of 10%CO_2, 7% O_2, and 83% N_2 at 5 psi, before harvesting and counting, and AFC quantified.

9. The results, depicted in Figure B below, showed that the immuno-stimulatory substance isolated from KCTC 0399BP resulted in a higher portion of antibody-forming B-lymphocytes than those isolated from the other *Phellineus linteus* strains as well as the controls.

B-lymphocyt s Activity f 0399BP (AFC assay)

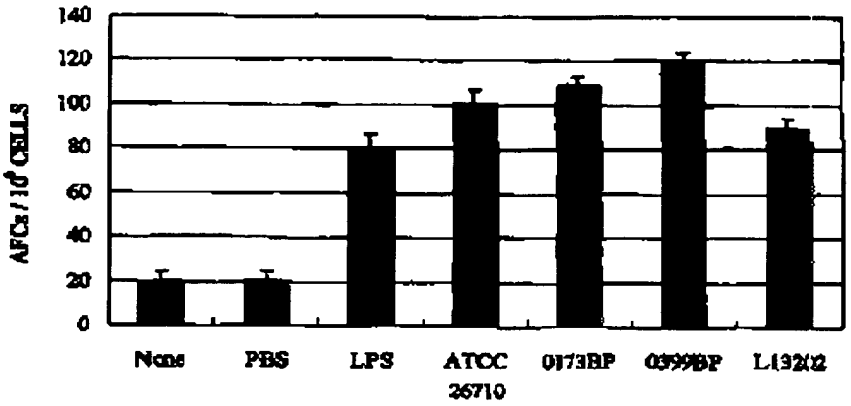

FIGURE B

10. I further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the instant application or any patent issued thereupon.

December 15, 2003
Dated:

Yoo Ick Dong
Ick-Dong Yoo